Capture of Mediterranean Fruit Flies (Diptera: Tephritidae) in Dry Traps Baited with a Food-Based Attractant and Jackson Traps Baited with Trimedlure During Sterile Male Release in Guatemala

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ABSTRACT Captures of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), in Jackson traps baited with trimedlure were compared with captures in cylindrical open-bottom dry traps baited with a food-based synthetic attractant (ammonium acetate, putrescine, and trimethylamine). Tests were conducted in Guatemala during a sterile male release program in an area where wild flies were present in low numbers. More wild and sterile females were captured in food-based traps, and more wild and sterile males were captured in trimedlure traps. The food-based traps captured almost twice as many total (male plus female) wild flies as the trimedlure traps, but the difference was not significant. Females made up $\approx\!60\%$ of the wild flies caught in the food-based attractant traps; the trimedlure traps caught no females. The ratio of capture of males in trimedlure traps to food-based traps was 6.5:1 for sterile and 1.7:1 for wild flies. Because fewer sterile males are captured in the food-based traps, there is a reduction in the labor-intensive process of examining flies for sterility. The results indicate that traps baited with food-based attractants could be used in place of the Jackson/trimedlure traps for C. capitata sterile release programs because they can monitor distributions of sterile releases and detect wild fly populations effectively; both critical components of fruit fly eradication programs by using the sterile insect technique.

KEY WORDS Ceratitis capitata, fruit fly trapping, synthetic food attractant, sterile insect technique

The Mediterranean fruit fly, Ceratitis capitata (Wiedemann), is a pest of major economic importance that threatens U.S. fruit and vegetable production and export. C. capitata is widely distributed throughout the world and has a host range of >300species of fruits and vegetables (Liquido et al. 1991, 1998). C. capitata has been introduced several times into the continental United States, sometimes resulting in breeding populations before successful eradication (Siebert and Cooper 1995, Hendrichs et al. 2002). Sterile insect technique (SIT) is a control method that has been used successfully for areawide population suppression and eradication of fruit flies (Gilmore 1989). The availability of temperature-sensitive lethal (tsl) genetic sexing strains of C. capitata makes it possible to rear and release a single sex (males), which further increases efficacy of SIT for C. capitata (Willhoeft et al. 1996). Current management

Approximately 50,000 traps are deployed in the United States for detection of C. capitata (Nilakhe et al. 1993). The Jackson trap (Harris et al. 1971) baited with trimedlure (Beroza et al. 1961), a parapheromone that attracts males primarily and is only weakly attractive to females (Nakagawa et al. 1970), is the trap used primarily for *C. capitata* detection. Jackson/trimedlure traps are used in conjunction with McPhailtype traps baited with liquid protein (Newell 1936), which capture both female and male flies. Trap density and the ratio of Jackson/trimedlure traps to McPhail/ protein traps vary with program strategy (Penrose 1993, 1996). During the 1989/1990 eradication program in California, five Jackson/trimedlure traps were placed per square mile in urban areas when used for detection surveys (Penrose 1993). If a single fly was detected, trap density was increased to 100 Jackson/ trimedlure traps and 25 McPhail/protein traps in the square mile that included the site of the find, with decreasing densities in the adjacent square miles for delimitation trapping. During the 1993/1994 program, detection surveys used 10 Jackson/trimedlure traps

strategies to diminish the threat of invasion of *C. capitata* include release of sterile male flies as part of preventative SIT programs (Hendrichs 1996), which are operational in California, Florida, and Texas.

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and five McPhail/protein traps per square mile, and a combination of yellow sticky panel/trimedlure traps, Jackson/trimedlure traps, and McPhail/protein traps were used for delimitation trapping (Penrose 1996).

Populations of *C. capitata* in Central America and Mexico are a threat to U.S. agriculture, and the USDA joined the Mexico-Guatemala Moscamed Commission (Moscamed) in 1977 to oversee a program to eradicate C. capitata from all three countries (Linares and Valenzuela 1993). As part of this barrier program, >13,000 Jackson/trimedlure traps were deployed in an area of >108,000 km2 in Guatemala (Linares and Valenzuela 1993) and >20,000 traps in Mexico (Antonio Villaseñor, personal communication). Much of this area is rugged, mountainous terrain that prevents the use of McPhail traps baited with aqueous protein solution, the standard female-biased trap recommended for use in *C. capitata* SIT (Katsoyannos 1994). With the development of a lure that combined ammonium acetate and putrescine, a synthetic attractant was available that provided the option of a dry femalebiased trap (Heath et al. 1995, Epsky et al. 1995). Field tests of cylindrical open-bottom dry (OBD) traps baited with ammonium acetate/putrescine and used with a sticky insert to retain attracted flies found that these dry food-based traps could be deployed in the mountainous, coffee-growing regions of Guatemala and that they were more sensitive to low numbers of wild flies than Jackson/trimedlure traps (Heath et al. 1996). Subsequent research found that trimethylamine synergizes capture of C. capitata in traps baited with ammonium acetate and putrescine in both OBD and McPhail-type traps (Heath et al. 1997). Although overall capture is better when ammonium acetate, putrescine, and trimethylamine lures are used in McPhail-type traps with water/surfactant versus the OBD traps (Epsky et al. 1999), the OBD/synthetic food-based lure traps are used currently in Guatemala and Mexico as part of the Moscamed program and are called phase 4 traps. Eradication and control efforts directed at established populations of C. capitata in Guatemala use aerial release of sterile male flies, and aerial and ground bait sprays (Orozco et al. 1994). Moscamed trapping protocols use variable combinations of traps, depending on SIT activity and projected monitoring needs. These range from all Jackson/trimedlure traps in areas monitored for future control efforts, to 4:4:2 OBD/food-based trap: Jackson/trimedlure:other trimedlure-baited traps outside of SIT release areas in coffee, to 9:1 OBD/food-based trap: Jackson/trimedlure trap in active release areas, at a density of ≈ 2 per km². The other trimedlure-baited traps include yellow panel traps and C & C traps (D.M., unpublished data; IAEA 2003).

Although the Jackson/trimedlure traps and the OBD/food-based traps are the standard traps used by Moscamed in Guatemala, additional information is needed on comparative performance of these trapping systems to make effective decisions in areas with breeding populations of *C. capitata* as well as in areas threatened by *C. capitata* invasion. Therefore, field

trials were conducted in Guatemala to compare captures of wild and sterile *C. capitata* between the OBD/ food-based traps and Jackson/trimedlure traps during SIT release.

Materials and Methods

Traps and Lures. Traps and lures were prepared following standard Moscamed protocol. Triangular cardboard Jackson traps were baited with the liquid trimedlure [tert-butyl-4 (and 5)-chloro-2-methylcyclo-hexane-1-carboxylate | (AgriSense BCS Ltd., Mid Glamorgan, United Kingdom). Trimedlure (2 ml) was applied to a cotton wick and mounted inside the trap. The traps contained white inserts that were coated on one side with sticky insect adhesive (Tangle Trap, Tanglefoot Co., Grand Rapids, MI) to retain flies attracted to the traps. OBD traps (Heath et al. 1996, IAEA 2003) were baited with a three-component food-based lure consisting of ammonium acetate, putrescine, and trimethylamine, formulated as three separate patches backed with adhesive for securing inside the trap (Suterra LLC, Bend, OR). The OBD trap (9 cm in diameter by 15 cm in height) was made locally from opaque green waxed cardboard and had three holes (2 cm in diameter) evenly spaced around the midline of the trap. A yellow sticky insert (7.6 by 12.7 cm; Suterra LLC) was hung inside the center of the trap to retain flies. Other studies have found that although the synthetic food-based lure can be used in Jackson traps for wild (R.R.H., unpublished data) or sterile flies (Montoya et al. 1999) or other delta traps (Tóth et al. 2004), capture of both wild and sterile males and females is lower in Jackson/food-based traps than in OBD/food-based traps (R.R.H. unpublished data; Montoya et al. 1999). Because of the desire by Moscamed program managers to optimize capture of wild female flies in a trap that can be deployed in the mountainous regions of Guatemala, only the OBD/food-based traps and Jackson/trimedlure traps were evaluated in this study.

Traps were serviced weekly at which time trimedlure was reapplied to the wicks in the Jackson traps and sticky inserts were replaced in both traps following the standard trapping protocol for the Moscamed program in Guatemala. The three-component synthetic lures were replaced every 4 wk, and trap bodies were replaced when the cardboard deteriorated and became deformed.

Fly Release. Sterile male-only *tsl* strain *C. capitata* (Vienna-7) adults were produced at the Moscamed rearing facility in El Pino, Guatemala. To obtain males only, eggs are placed in warm baths (34°C) to kill females that have the *tsl* gene (Franz et al. 1996). Larvae are reared on a diet consisting principally of 15% sugarcane bagasse, 9% yeast, 15% sugar, and water. Pupae are sterilized with 10 Krad (100 Gy) of gamma radiation from Husman (Cesium 137) or Gamacell (Cobalt 60) irradiators. The irradiated pupae are treated with 4 g/liter of powdered fluorescent dye (Dayglo Color Corp., Cleveland, OH) to mark the

adults on emergence (Steiner 1965). The pupae are transported to the emergence facility in Retalhuleu, Guatemala, where they are kept at a temperature of 23°C and fed a mixture of 15% sugar and 84.99% water thickened by 0.01% agar (Jose Ponciano, personal communication). At 3–4 d of age, the flies are chilled to near 0°C and loaded into chilled-fly release machines (K&K Aircraft, Bridgewater VA) installed in a Cessna Caravan. The sterile flies were applied to the study area from an altitude of 2,500–3,000 m above sea level. Sterile flies were released weekly at an average rate of 3,600 flies per hectare.

Field Protocol. The study was conducted in a geographically diverse area of Guatemala under SIT release by the Moscamed program. The field site was \approx 20 km² and was centered at longitude -91.53° and latitude 14.71° in the Santa María valley between the Santa María and Santo Tomas volcanoes. Trap sites were determined by generating a grid of potential trap locations based on a point in the center of the valley and spaced evenly 500 m apart. Traps were then placed as close to these potential trap locations as possible using a global positioning system (Garmin GPS III Plus, Garmin International Inc., Olathe, KS). Once a site was chosen, traps were placed in pairs (one OBD/food-based and one Jackson/trimedlure) ≈25 m apart in trees of the same type. Following standard trapping recommendations (IAEA 2003), most traps were placed in or near host trees, primarily coffee or shade trees within a coffee plantation or, if no host trees were near the prospective location, a trap was placed in a nonhost tree. The trap locations varied in elevation from 1000 to 1,800 m above sea level. The traps were serviced weekly for nine consecutive weeks from June to September 2002. All trap inserts were taken to the Moscamed laboratory in Mazatenango, Guatemala where the Moscamed laboratory personnel determined the number, sex, and sterility of captured flies following standard protocols (Guillen 1983).

Data Analysis. The data from the 51 trap sites (51 pairs of the two trap types) were collected for 9 wk, resulting in a total of 459 paired observations. Numbers of flies captured per trap per week were averaged over the 9 wk and the average capture used for subsequent analysis (n = 51). Separate analyses were conducted for captured wild male, female and total (male plus female) flies, and sterile male and female flies. Homoscedasticity was not reached after several transformations; therefore, a Wilcoxon two-sample test was used to compare the number of flies captured in the two treatments (PROC NPARIWAY, SAS Institute 1998). Average capture for each week of the study was used to determine 1) the ratio of sterile males and wild males in the paired trapping systems and correlation of these captures within each fly group (sterile or wild males) by using the Spearman's correlation coefficient (PROC CORR, SAS Institute 1998) and 2) the percentage of females among the wild flies captured in the OBD/food-based traps.

Table 1. Mean (\pm SEM) capture per trap per week of *C. capitata* in Jackson/trimedlure and OBD/food-based attractant (ammonium acetate, putrescine, and trimethylamine) traps in the Santa María region of Guatemala (n=51)

Sterility and sex	Jackson trap with trimedlure	OBD trap with food-based lure	Z	P
Wild males	0.8 ± 0.14	0.6 ± 0.18	3.0143	0.0013
Wild females	0.0 ± 0.00	0.9 ± 0.21	6.0774	< 0.0001
Total wild flies	0.8 ± 0.14	1.6 ± 0.46	0.8852	0.1880
Sterile males	111.0 ± 5.39	17.6 ± 1.32	6.6126	< 0.0001
Sterile females	0.0 ± 0.00	0.1 ± 0.02	5.3643	< 0.0001

Wilcoxon two-sample test used for mean comparison.

Results and Discussion

Of the 60,810 flies (sterile and wild) captured during the experiment, 51,948 were found in Jackson/trimedlure traps and 8,862 in OBD/food-based traps. Table 1 presents the average number of flies captured per week over the whole study. Jackson/trimedlure traps captured more wild and sterile males than OBD/food-based traps, and OBD/food-based traps captured more wild and sterile females than the Jackson/trimedlure traps. Although OBD/food-based traps captured twice as many total (male plus female) wild flies, the difference was not significant. Sterile males captured by the Jackson/trimedlure trap accounted for ~85% of all of the flies captured during the experiment.

Numbers of sterile and wild flies captured were determined at the laboratory in Mazatenango, Guatemala, where hundreds of traps were processed daily. During this processing, flies are handled in four different steps (Villaseñor and Hernández 1990). First, the flies are placed on grid paper and examined under UV light for presence of fluorescent dye on the head or body of the fly. Any flies not obviously marked are sent to the second screening step in which the heads are crushed on filter paper and reexamined under UV light. If no marking is detected, they are then examined by fluorescent microscopy for presence of dye. If still negative, they are subjected to a final step that consists of dissecting the abdomen and examining the testes to determine fertility (Anwar et al. 1971, Guillen 1983). Although information on numbers of sterile flies captured in our study that required dissection was not recorded, ≈3% of the sterile males brought in for assessment of sterility advance to the second step and. although very few unmarked flies are determined to be fertile in the final step (≈ 1 in 50,000), the entire examination process constitutes a significant amount of labor (Enkerlin et al. 1996). Generally, in an 8-h day, technicians can identify sterility of $\approx 1,500$ flies. This is equivalent to 0.32 min per fly. Therefore, a Jackson/ trimedlure trap that captures 100 flies is processed in 32 min, whereas an OBD/food-based trap that captures 16 flies is processed in ≈5 min. This reduction in handling would result in a reduction in labor time and costs. Expanded to include all release blocks within the regional Moscamed Program in Mexico and Guatemala, the cost savings would be considerable. In addition, the fatigue experienced by laboratory work-

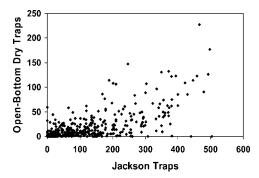


Fig. 1. Number of sterile male C. capitata captured per trap per week in Jackson traps baited with trimedlure plotted against number captured in paired OBD traps baited with a food-based synthetic attractant (ammonium acetate, putrescine, and trimethylamine) in field trials conducted in the Santa María region of Guatemala (n=461).

ers processing large numbers of sterile flies would be reduced. The reduction in workload would allow greater attention to each fly and likely result in fewer misidentifications (unmarked wild flies identified as sterile, or vice versa). Fewer flies in the traps will reduce the possibility that wild flies are "falsely" marked while removing the sterile-marked ones from the traps.

One of the main purposes of trapping during SIT programs is to monitor the distribution of sterile insects. A scatter plot of the data for all weeks is presented in Fig. 1. Correlations were found between the capture of sterile males in the Jackson/trimedlure and the OBD/food-based traps for each week of the study (Table 2). There were 6.5 ± 0.42 (mean \pm SEM) sterile males captured in Jackson/trimedlure traps for every one sterile male captured in the OBD/foodbased trap, with the numbers ranging from 5.2:1 to 9.3:1 over the 9 wk of the study. Despite enormous efforts to release the flies in a uniform manner throughout the target area, their ultimate distribution is nonuniform due to factors such as weather, topography, host distribution, and bodies of water. Therefore, a reliable trapping system is required to inform program managers of the actual spatial distribution of

Table 2. Mean (\pm SEM) weekly capture of sterile male *C. capitata* in Jackson/trimedlure or OBD/food-based attractant (ammonium acetate, putrescine, and trimethylamine) traps and correlation of this capture between the paired trap/lure treatments (n=51)

Wk	Jackson trap with trimedlure	OBD trap with food-based lure	Spearman rho	P
1	110.9 ± 14.84	11.9 ± 2.39	0.49874	0.0002
2	119.9 ± 15.14	16.9 ± 3.33	0.42915	0.0017
3	115.7 ± 14.76	16.4 ± 3.28	0.59824	< 0.0001
4	111.2 ± 17.42	20.4 ± 5.30	0.70263	< 0.0001
5	132.1 ± 17.39	23.5 ± 5.76	0.70813	< 0.0001
6	123.6 ± 18.20	23.7 ± 4.56	0.76534	< 0.0001
7	94.3 ± 16.22	15.0 ± 3.07	0.57039	< 0.0001
8	98.9 ± 16.41	14.0 ± 3.20	0.79069	< 0.0001
9	104.4 ± 15.66	16.8 ± 3.48	0.75607	< 0.0001

Spearman's correlation coefficient analysis.

the sterile release. However, accurate information about the sterile fly distribution does not require a trapping method that captures large numbers of flies. In our study, the OBD/food-based traps captured significantly fewer sterile *C. capitata* than the Jackson/trimedlure traps, but these captures were correlated with the captures in Jackson/trimedlure traps.

It might be argued that the decreased number of sterile male captures in the OBD/food-based traps will not provide the same detail of density information as the Jackson/trimedlure traps, thus reducing their utility in evaluating the distribution of the sterile flies. However, if the objective of monitoring the distribution is to ensure that sufficient numbers of males are reaching all areas, then the lower threshold of the detection in the OBD/food-based trap is more than sufficient. A single capture in the OBD/food-based trap averaged 6.5 flies in the Jackson/trimedlure trap. This number is below the minimum threshold of 21 sterile flies per week (three flies per day) in Jackson/ trimedlure traps located within release blocks as determined by managers of Moscamed in Guatemala and Mexico. A Jackson/trimedlure trap within a release block that captures <21 flies or an OBD/food-based trap that captures less than three flies are both interpreted in the same way: an extremely poor density of the flies in that portion of the release block. This threshold may be adjusted depending on the density of flies released and the local situation for other C. capitata sterile release programs; however, the threshold of detection (one fly per week in OBD/food-based trap or 6.5 flies per week in Jackson/trimedlure trap) should be sufficient for most uses.

Use of the OBD/food-based trap provided a good measure of the distribution of the sterile flies and provided improved capture of wild flies because both male and female flies were captured in these traps. The ratio (mean ± SEM) of capture of wild males in Jackson/trimedlure to wild males in OBD/food-based trap was 1.7 ± 0.21 , ranging from 0.75 to 3.0 per week (Table 3). There were correlations between wild male capture in the two trap/lure types for 8 of 9 wk of the study, although the correlations were not as strong as those observed with sterile male capture. The difference in average response of sterile males (6.5:1) versus response of wild males (1.7:1) to the two trap/lure combinations may indicate a greater responsiveness of sterile males to trimedlure or of fertile males to foodbased attractants. Sterile males are given access to sugar before field release and may have less of a requirement for food due to their sterility status, and thus may be less responsive to food-based lures. Sexual maturity of the flies also may be a factor. Sterile males are sexually mature when released, whereas wild males will be a mix of sexually immature and mature individuals. Studies in Hawaii found no difference in response of wild males versus sterile males to trimedlure (McGovern et al. 1987), but no information on age or handling of sterile males was given. In release– recapture comparisons of Jackson/trimedlure traps and McPhail/food-based traps baited with liquid protein, however, Bloem et al. (1993) found differences

Table 3. Mean (±SEM) weekly capture of wild *C. capitata* in paired Jackson/trimedlure or OBD/food-based (ammonium acetate, putrescine, and trimethylamine) traps and correlation of male capture in the paired trap/lure treatments (n = 51)

Wk	Male capture		0 1	n	Female capture	
	Jackson trap with trimedlure	OBD trap with food-based lure	Spearman rho	P	OBD trap with food-based lure	
1	1.4 ± 0.55	1.9 ± 1.18	0.48504	0.0003	2.8 ± 1.56	
2	1.5 ± 0.55	0.9 ± 0.42	0.40696	0.0030	1.8 ± 0.61	
3	0.7 ± 0.22	0.3 ± 0.13	0.54503	< 0.0001	0.7 ± 0.28	
4	0.5 ± 0.13	0.2 ± 0.09	0.37840	0.0062	0.3 ± 0.12	
5	0.8 ± 0.29	0.3 ± 0.13	0.25653	0.0692	0.2 ± 0.12	
6	0.2 ± 0.06	0.1 ± 0.05	0.47112	0.0005	0.2 ± 0.10	
7	0.7 ± 0.26	0.5 ± 0.28	0.34505	0.0131	0.8 ± 0.37	
8	0.9 ± 0.31	0.6 ± 0.37	0.31244	0.0256	0.7 ± 0.47	
9	0.9 ± 0.32	0.7 ± 0.40	0.46187	0.0006	0.8 ± 0.40	

No wild females were captured in Jackson/trimedlure traps. Spearman's correlation coefficient analysis.

between response of sterile flies versus wild. In field test comparisons of capture in Jackson/trimedlure traps and OBD/food-based traps, ratio of wild males in the two traps ranged from ≈ 2.1 to ≈ 39.1 (Epsky et al. 1999). In these studies, the lowest ratio corresponded to population levels of ≈2 males per Jackson/trimedlure trap per week, whereas the highest ratio corresponded to population levels of ≈64 males per Jackson/trimedlure trap per week. Although it was not evaluated in our study, previous studies have compared capture of C. capitata in Jackson or Jackson-type delta traps baited with a three component synthetic food-based lure. In field tests of capture of wild flies conducted in Italy, ratio of males and females in Jackson-type/trimedlure traps versus Jackson type/foodbased traps varied from ≈5:1 to ≈20:1 and no capture to 0.08:1, respectively (Tóth et al. 2004). These ratios are within the range obtained in studies reported in Epsky et al. (1999). In field tests of ground release of sterile bisexual-strain *C. capitata* conducted in Mexico (Montoya et al. 1999), ratio of males and females in Jackson/food-based traps versus OBD/food-based traps was 1.08:1 and 0.50:1, respectively.

Data on the wild population are a critical component for eradication efforts and OBD/food-based traps caught nearly double the number of wild flies as the Jackson/trimedlure traps. Notably, $59.1 \pm 3.04\%$ (mean ± SEM) of the wild flies captured by the OBD/food-based traps were female, with the percentage ranging from 40 to 70% over the 9 wk of the test (Table 3). Detection of wild females provides important information about the potential location of breeding populations, and female captures are weighted more heavily within the Moscamed protocol for categorizing locations of outbreaks and deciding on areas for treatment (D.M., unpublished data). In addition, it has been shown that female-biased C. capitata traps detect populations at lower levels and earlier than Jackson/trimedlure traps (Papadopoulos et al. 2001, 2003). Early detection of reproducing populations in low prevalence areas is essential for successful eradication. A delay of a few weeks could result in large increases in time, effort, and money to control insipient outbreaks.

A key objective of trapping is to compare the number of released flies to the number of wild flies within the SIT target area. Jackson/trimedlure traps captured large numbers of sterile males, few wild males, and no females. The basis of Knipling's SIT procedures is the sterile/wild fly overflooding ratio (Knipling 1955). The Moscamed programs in Mexico and Guatemala have set a target ratio of 80-100 sterile males to one wild fly, of either sex (Villaseñor and Hernández 1990). Our data indicate that 100 sterile male flies in a Jackson/trimedlure trap are equivalent to 15–16 sterile male flies in an OBD/food-based trap. Additional studies are required to adjust the overflooding ratio based on OBD/food-based traps, because they catch a proportionally larger pool of wild flies than Jackson/ trimedlure traps. In this study, the proportion of wild versus sterile flies captured by OBD/food-based traps (8.8%) was an order of magnitude higher than that in the Jackson/trimedlure traps (0.74%). Even if only male flies are considered for calculation of the overflooding ratio (male to male competition) the proportion of wild to sterile males captured is still nearly 5 times greater (3.5%) in the OBD/food-based traps. This differential sampling of the population by the two traps is potentially important and needs further study. The 100:1 overflooding ratio is based on mathematical models and the calculation of release density for sterile flies assumes that the trapping data are a reliable indicator of the size of the fly population. Capture in OBD/food-based traps provides information on both male and female wild flies as well as on sterile flies. Incorporation of information about the density of both wild male and female fly populations into the SIT models may contribute to a better assessment of the sterile fly density required per unit surface to effectively achieve the desired sterile to wild ratio, thus improving the effectiveness of the sterile release programs for C. capitata.

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